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CONTENT OF SELECTED ANTIOXIDATIVE COMPOUNDS IN GREEN ASPARAGUS DEPENDING ON PROCESSING BEFORE FREEZING AND ON THE PERIOD AND CONDITIONS OF STORAGE

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The content of antioxidative compounds was evaluated in frozen green asparagus produced with the traditional technology from the material blanched before freezing or with the modified technology from cooked asparagus. Compared with blanched asparagus, the product cooked before freezing contained more dry matter and polyphenols, similar amounts of beta-carotene, less carotenoids and vitamin C and its antioxidative activity was lower. During a 12-month storage at -20°C and -30°C a steady decrease in the level of the analysed constituents was observed in frozen products prepared for consumption. Compared with the raw material, asparagus prepared for consumption after the 12-month period of refrigerated storage contained 56–62% of vitamin C; 55–71% of polyphenols; 73–81% of beta-carotene; 74–89% of carotenoids while its antioxidative activity was reduced to 65–73%. In products obtained using the modified method the level of the analysed constituents was similar or a little higher than in the traditional products. Frozen products stored at -30°C were usually characterised with a higher content of analysed constituents and a higher level of the antioxidative activity in comparison with frozen asparagus stored at -20°C. Sensory quality of traditional frozen products slightly exceeded that of frozen products obtained using the modified method. The quality of products stored at -30°C was also better than that of products stored at -20°C.

INTRODUCTION

In recent years the interest of consumers in health-promoting food products has been observed to increase. This is associated with the quest for rational methods of preventing diseases and improving the resistance of human organisms [Grajek, 2004]. Vegetables which are important constituents of our diet can be classified as such products owing to their high nutritive values. They supply constituents significant for the proper functioning of our organisms. Many of these substances take part in processes preventing or limiting the oxidation of cell constituents, protecting our organisms against degeneration diseases [Bazzano *et al*., 2002].

Comparing with other European countries the consumption of both fresh and processed vegetables is too low in Poland [Szponar *et al*., 2003], being below 75% of the amount of fruit and vegetables recommended in a model of rational nutrition [WHO, 2003]. The limiting factor can be the poor assortment of fresh and processed vegetable products in the market, being most often limited to such vegetable species as cabbage, carrot, red beet and cauliflower. Thus it is deemed appropriate to introduce vegetable species known in other European countries into the production and processing industry. Asparagus, highly valued for their taste, can be quoted as an example of such vegetables [Pellegrini *et al*., 2003; Shao *et al*., 1996]. White asparagus is popular in Italy, Spain and the Netherlands while in Denmark green asparagus covers over 60% of the cultivation area [Kidmose & Kaack, 1999]. In Poland the season of supply of fresh asparagus is fairly short. Since the storage life of this vegetable is short its availability in the market can be lengthened by the supply of frozen and canned products [Fuleki, 1999; Sun *et al*., 2005].

The increasing widespread interest of consumers of processed food concerns products of the ready-to-eat type which can be prepared for consumption by rapid heating, for example in a microwave oven. Such are frozen products prepared from vegetables cooked before freezing.

The aim of the present work was to evaluate the content of selected antioxidative compounds in the raw material, in blanched asparagus; cooked asparagus; and in frozen products prepared for consumption while obtained using the traditional technology of blanching the raw material or the modified technology of cooking it. The evaluation was conducted after 0, 4, 8 and 12 months of refrigerated storage at -20°C and -30°C.

MATERIAL AND METHODS

MATERIAL

The investigated material was the above-ground shoots of green asparagus. The investigation covered fresh shoots

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of asparagus; shoots prepared for freezing (blanched or cooked) and frozen shoots directly after freezing and after 4-, 8- and 12-month periods of refrigerated storage at -20°C and -30°C; after each period the analysed product was prepared for consumption.

The cultivar used in the investigation was Thielim (Asparagus BV) recommended for processing, a medium-early cultivar, adapted to the conditions of temperate climate. The raw material was obtained from a specialized farm located in mid-eastern Poland, in the region of Kazimierz Dolny (Lublin province). The harvest and processing of asparagus were carried out early in June. Asparagus shoots were harvested at evening hours on the day preceding technological processing and evaluation of the raw material. Fresh shoots were transported and stored in an isothermal container cooled to 6°C. Before processing asparagus shoots were washed and sorted to select these 12–20 mm in width. Then they were cut into pieces 35–40 mm in length. The remaining material was divided into two parts, each being processed using a different technology of preparation for freezing and preparation of frozen products for consumption.

METHODS

Freezing of asparagus. Two variants of processing the raw material before freezing were applied. Variant I followed the traditional method of blanching before freezing. After freezing and refrigerated storage the product had to be cooked before consumption. In variant II the material was cooked to a consistency approximating to consumption consistency before freezing, yielding the product of the ready--to-eat type which merely required defrosting and heating in a microwave oven.

Parameters of thermal processing. In variant I cut asparagus shoots were blanched in a stainless steel vessel in water, the proportion of water to material being 5:1 by weight, at 96-98°C for 4 min. The conditions of blanching were regulated so as to decrease the activity of catalase and peroxidase to a level not exceeding 5% of the initial activity. After blanching the material was cooled in cold water and left to drip on sieves for 30 min. In variant II asparagus shoots were cooked in a stainless steel vessel in water with 2% added salt, the proportion of water to material being 1:1 by weight. The material was put in boiling water, the time of cooking of 5 min 30 sec being measured from the moment when the water came to the boil again. After cooking the material was left on sieves and cooled in a stream of cold air.

The material consisting of blanched and cooked samples was divided each into two parts; placed on trays and frozen at -40°C in a Feutron 3626-51 blast freezer. One part of the material was frozen to a temperature of -20°C for 90 min and the other to -30°C for 120 min. Frozen products were packed in 500 g polyethylene bags suitable for the storage of frozen vegetables; then left in chamber freezers respectively at -20°C and -30°C; and stored until evaluation.

Preparation of frozen asparagus for evaluation. Frozen products obtained using traditional method (variant I) were cooked in water with 2% added salt, the proportion of water to the material being 1:1 by weight. Frozen asparagus was put in boiling water, the time of cooking of 3 mins 30 sec being measured from the moment when the water came to the boil again. After cooking, the water was drained and the product cooled to 20°C; then its chemical composition was evaluated.

Frozen samples of cooked asparagus (variant II) were defrosted and heated in a Panasonic NN-F621 microwave oven to 75°C [Codex Alimentarius, 1993], for 7 min 45 sec in the case of samples stored at -20°C or for 8 min 15 sec in the case of samples stored at -30°C. After heating the products were cooled to 20°C and their quality was evaluated.

Evaluation of the chemical composition and sensory estimate of the products. Dry matter content was determined by means of gravimetry as the mass loss of a sample at 96–98°C [AOAC, 1984]. The content of vitamin C was determined as the sum of ascorbic acid and dehydroascorbic acid by method described by Gil *et al*. [1999]. The analysis was performed with the HPLC using a Merck-Hitachi system with a UV detector. Ascorbic acid and dehydroascorbic acid were quantified with a C18 column with a mobile phase composed of 5% methanol in water solution containing 50 mmol/L KH_2PO_4. The detection was made at 261 nm for ascorbic acid and for 348 nm dehydroascorbic acid after derivatization with 1,2-phenylenediamine.

Total carotenoids were determined using the spectrophotometric method after extraction with acetone [Lichtenthaler & Buschmann, 2001]. Beta-carotene analysis [ISO, 1992] consisted of the extraction procedures of pigment, followed by liquid/liquid partitioning with hexane, concentration and column chromatography. The same extracts obtained for carotenoids estimation were used.

Total phenolic compounds were determined using the Folin-Ciocalteau reagent [Singleton *et al*., 1999]. The absorbance was measured at 760 nm in a Shimadzu UV-VIS 160A spectrophotometer using chlorogenic acid as a standard. The antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method [Pekkarinen *et al*., 1999] in ethanolic extracts prepared by heating (for 20 min) homogenized samples with 80% alcohol under reflux. The antioxidant activity was determined by measuring the absorbance at 516 nm in the above described spectrophotometer and expressed as % RSA (Radical Scavenging Activity). The content of the analysed constituents was compared in the raw material, semi-products and frozen vegetables prepared for consumption and their level was expressed in 100 g fresh matter, that is, in 100 g of the raw material or the product. The content of dry matter, for each stage of evaluation, allows the reader to calculate the presented results to dry matter. All analyses were done in four replications.

Since one of the objects of the experiment consisted of products of a new ready-to-eat type, it was deemed appropriate to compare the sensory traits of frozen asparagus prepared according to the traditional and modified variants after the 12-month storage period. The evaluation in a scale from 1 to 5 was carried out by a team of five panelists who met the basic requirements of sensory sensitivity [ISO, 1991] in conditions conforming to ISO [1985], using a model chart devised by the present authors. The sum product of sensory evaluation points multiplied by their weighing factors was divided by the total weighing factors, and this was accepted as a total score.

In order to show the differentiation in chemical composition and sensory evaluation a one-way analysis of variance (ANOVA) was carried out on the basis of the Snedecor's F-test and the Student's t-test, the least significant difference (LSD) for the error probability level being calculated at $\alpha=0.01$ for the chemical composition and $\alpha=0.05$ for sensory evaluation. The computer program Statistica ver.6.1 was used for calculations.

RESULTS AND DISCUSSION

The content of the analysed constituents such as dry matter, vitamin C, carotenoids and polyphenols in fresh material was similar or slightly lower (Table 1) than that reported by different authors for asparagus [Deli *et al*., 2000; Kmitiene, 2004; Vinson *et al*., 1998]. Using the content of dry matter and vitamin C as examples Kmitiene [2004] and Lee & Kader [2000] stress that the content of various constituents in vegetables depends on numerous factors, such as the variety factor or the conditions of storage after harvest. Asparagus is classed in the group of vegetables of a high antioxidative activity [Nindo *et al*., 2003; Vinson *et al*., 1998]. The analysed asparagus shoots were characterised by higher antioxidant activity than broccoli [Gębczyński & Lisiewska, 2006]. Rodriguez *et al*. [2005] also found that comparing with the cultivars of white shoots these of coloured shoots, including a number green ones, were characterised by a higher antioxidative activity.

Blanching of the raw material before freezing significantly decreased the level of the analysed traits including the antioxidative activity (Table 1). The recorded loss varied from 4% for carotenoids to 16% for antioxidative activity. No changes were found in the level of dry matter. On the other hand the cooking of asparagus before freezing brought about a 14%

TABLE 1. Contents of analysed components in fresh green asparagus and asparagus prepared for freezing.

Analysed components	Fresh asparagus	Asparagus prepared for freezing		LSD
		blanched	cooked	$\alpha=0.01$
Dry matter (g/100 g)	6.49 ± 0.02	6.47 ± 0.01	7.38 ± 0.01	0.18
Vitamin C (mg/100 g)	19.5 ± 0.61	16.9 ± 0.51	15.8 ± 0.52	1.23
Total polyphenols (mg/100 g)	95.6 ± 1.30	74.3 ± 1.20	84.8 ± 1.30	2.26
Total carotenoids (mg/100 g)	2.09 ± 0.04	2.00 ± 0.04	1.90 ± 0.05	0.089
β-Carotene (mg/100 g)	0.63 ± 0.02	0.57 ± 0.02	0.59 ± 0.02	0.051
Antioxidant activity (% RSA)	55.6 ± 0.60	46.8 ± 0.30	45.5 ± 0.60	1.14

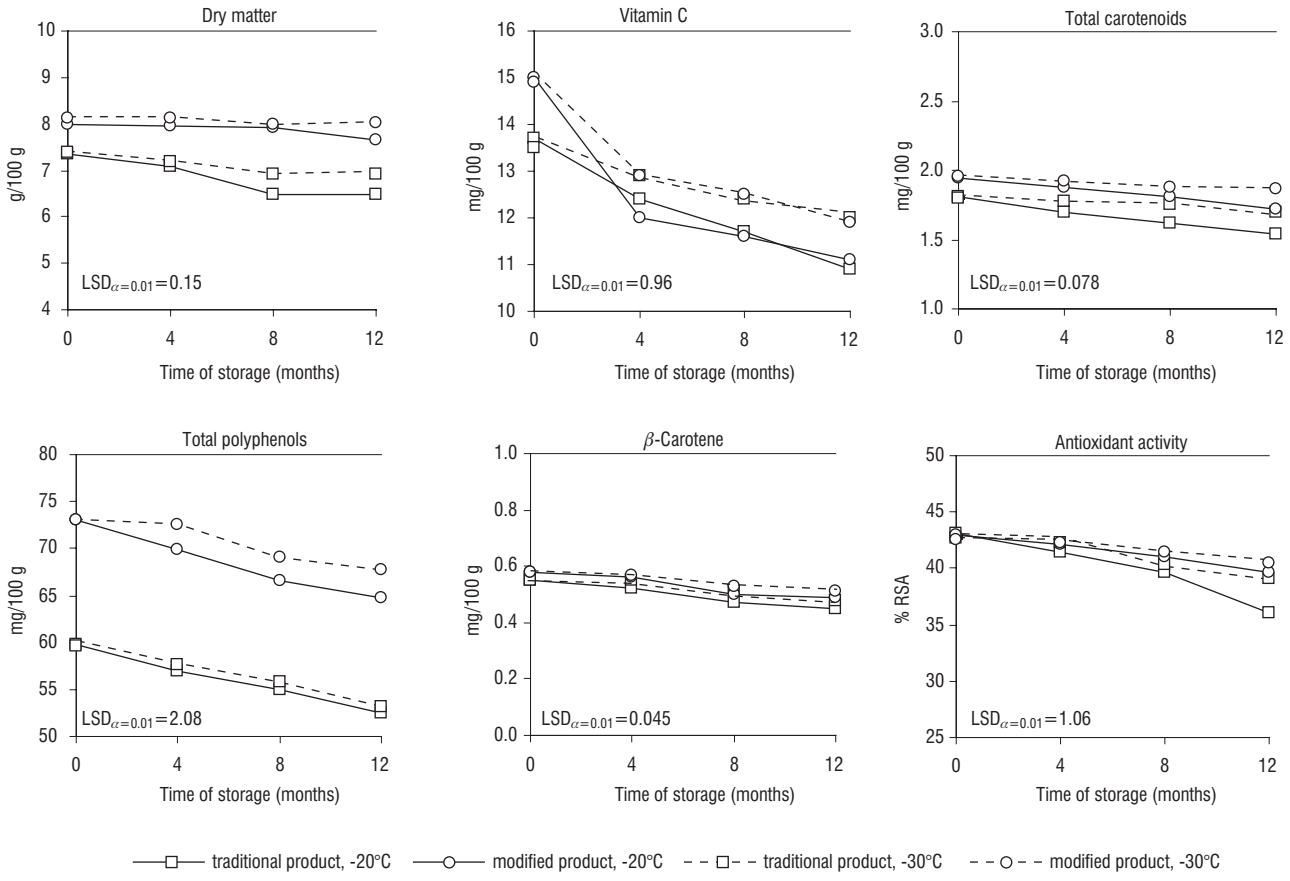

FIGURE 1. Changes of the analysed components and antioxidant activity in frozen green asparagus stored at different temperatures, and then prepared for consumption.

increase in this level. An increase in the level of dry matter was caused by the absorption of salt from brine. This could have been associated with a release of water from the plant tissue. Since asparagus is a vegetable of high water content and a delicate structure, the release of water could have been expected during thermal processing. Kidmose & Kaack [1999] confirm this supposition, reporting an increased loss of dry matter in asparagus with the lengthened time of blanching. Compared with blanching, greater decreases in the content of vitamin C and carotenoids, similar changes in the content of beta-carotene and in the antioxidative activity; and a smaller loss of polyphenols were found during cooking. When compared with the raw material, in asparagus cooked before freezing the recorded losses were: 19% in vitamin C content; 9% in carotenoids; 6% in beta-carotene; 11% in polyphenols; and 18% in antioxidative activity. Changes which occur in the content of chemical constituents during thermal processing in a water environment are evoked by their thermal and enzymatic degradation or by leaching [Petersen, 1993; Lee & Kader, 2000]. The amount of losses of many constituents is also associated with the size of particles and the degree of grinding of the vegetable. In blanched leaf vegetables and cooked pea and cubed kohlrabi the loss of vitamin C reached 60% [Selman, 1994]. The described phenomenon is also affected by changes in the weight of the material which above all depend on the release and absorption of water by plant tissues [Sà & Rodriguez-Amaya, 2004].

The freezing of asparagus both to a temperature of -20°C and of -30°C and then the preparation of frozen products for consumption by cooking in brine or heating in a microwave oven brought about a significant 13–25% increase in dry matter content and significant decreases in the level of the remaining constituents and antioxidative activity (Figure 1). Compared with the raw material the content of vitamin C was reduced by 23–30%; that of polyphenols by 24–38%; that of carotenoids by 6–13%; and that of beta-carotene by 8–13%, whereas the antioxidative activity decreased by 22–24% in frozen products evaluated directly after freezing.

In further stages of the investigation, which covered the evaluation of frozen products prepared for consumption after 4, 8 and 12 months of refrigerated storage, no significant changes were found in the content of dry matter, showing that the investigated cultivar of asparagus was well suited to the long-term refrigerated storage. Neither did Kidmose & Kaack [1999] record changes in dry matter content of green asparagus after its 6-month storage at -20°C.

In the course of refrigerated storage of asparagus steady decreases were noted in the level of the investigated increments. However, not all the differences between the periods of analyses were statistically significant. In general, statistically significant differences in the content of vitamin C, polyphenols, carotenoids and beta-carotene and in the antioxidative activity occurred as late as after 8 months of storage. After 12 months of storage, depending on the investigated sample, decreases in vitamin C content reached 11–26%; in polyphenols 7–12%; in carotenoids 5–15% and in beta-carotene 12–16%; whereas decreases in the level of antioxidative activity varied between 5–16%. Irrespective of the method of production and preparation of frozen asparagus for consumption a better retention of the investigated characteristics was found in samples stored at -30°C compared with those stored at -20°C. When the technology of production of frozen asparagus is taken into consideration a better retention of polyphenols, carotenoids and of the antioxidative activity was found in products obtained using the modified method when compared with traditional products. However the content of vitamin C was similar in both types of frozen products.

Compared with the raw material and depending on the investigated sample frozen shoots of green asparagus prepared for consumption after 12 months of frozen storage contained 56–62% of vitamin C; 55-71% of polyphenols; 74–89 carotenoids and 73–81% of beta-carotene. Negative effects of the storage period on the level of the analysed constituents in frozen vegetables have also been reported by other investigators [Gębczyński, 2003; Jaworska & Kmiecik, 2000; Kidmose & Kaack, 1999; Pupponen-Pimiä *et al*., 2003]. However, the above-mentioned authors evaluated frozen products only after defrosting hence it is difficult to compare their results with these found in the present investigation. In the products prepared for consumption after 12 months the level of the antioxidative activity was 65–73% of that found in the raw material. Pupponen-Pimiä *et al*. [2003] also observed decreasing values of the DPPH index during the refrigerated storage of broccoli and pea. As Makris & Rossiter [2001] stress in processing raw plant materials the decreasing level of antioxidative activity can be associated both with the loss of substances of antioxidative capacity and with the appearance of substances of pro-oxidative properties.

Frozen products stored for 12 months were characterized with sensory quality at a good level at least. In a scale from 1 to 5 the final appraisal of frozen products evaluated before defrosting was 4.47–4.84 (Table 2) and of cooked frozen products 4.09–4.95 (Table 3). In evaluating the frozen products before defrosting and products after preparing for consumption higher quality was observed for these obtained from the material blanched before freezing chiefly owing to a better retention of the green colour. In products obtained from cooked asparagus the colour was of green-olive or olive shade. Decreases in the total scores found in products prepared for consumption were connected with unfavourable changes in colour and in the appearance of the surface of particles. In the latter case it was connected with the appearance of wrinkles on some parts of asparagus which could be observed especially in samples defrosted and heated in the microwave oven. Compared with samples stored at -20°C

TABLE 2. Sensory evaluation of frozen products after 12-month storage.

Quality and weight factors		Traditional product [a]		Modified product [b]	
		-20°C	-30°C	-20°C	-30°C
Frosting and conglomerates	2	4.6	4.6	4.6	4.6
Surface appearance	3	5.0	5.0	4.5	4.5
Colour	6	4.7	5.0	4.1	4.2
Consistency	3	5.0	5.0	5.0	5.0
Flavour	6	4.6	4.6	4.6	4.6
Total score		4.75	4.84	4.47	4.52
LSD[c] $\alpha = 0.05$		0.081			

[a] frozen product manufactured according to traditional procedure
[b] frozen product manufactured according to modified procedure
[c] LSD value for total score

TABLE 3. Sensory evaluation of frozen products prepared for consumption after 12-month storage.

Quality and weight factors		Traditional product [a]		Modified product [b]	
		-20°C	-30°C	-20°C	-30°C
Surface appearance	2	4.8	4.8	3.9	4.0
Colour	5	4.8	5.0	3.9	4.1
Consistency	3	4.6	4.8	4.2	4.5
Flavour	5	5.0	5.0	4.3	4.5
Taste	5	4.8	4.8	4.2	4.4
Total score		4.81	4.95	4.09	4.33
LSD[c] $\alpha = 0.05$			0.122		

[a] frozen product manufactured according to traditional procedure and cooked before consumption

[b] frozen product manufactured according to modified procedure and microwaved before consumption

[c] LSD value for total score

these stored at -30°C showed significantly higher sensory quality with the exception of frozen products from cooked asparagus evaluated before defrosting. In the case of frozen samples before defrosting the discussed quality differences were connected with a better retention of colour of asparagus; in the case of products obtained using the traditional technology and prepared for consumption with a higher evaluation of colour and consistency; and of products obtained according to the modified technology with a higher evaluation of all the characteristics.

CONCLUSIONS

The cooking of green asparagus before freezing evoked a 19% decrease in the content of vitamin C; an 11% decrease in polyphenols; a 9% decrease in carotenoids and a 6% decrease in beta-carotene, the antioxidative activity being reduced by 18%. Compared with blanched asparagus, cooked products contained more polyphenols; a similar quantity of beta-carotene but lower amounts of carotenoids and vitamin C; and their antioxidative activity was reduced. When compared with the evaluation carried out directly after freezing, a 12-month period of refrigerated storage brought about decreases in all the samples on the average: a 22% decrease in vitamin C content; 9% in polyphenols; 14% in beta-carotene; and 9% in antioxidative activity. Compared with the frozen product obtained using the traditional technology, frozen products of green asparagus obtained using the modified technology and prepared for consumption after 12 months of storage showed a similar level of vitamin C and beta-carotene, contained 25% more polyphenols, 10% more carotenoids and their antioxidative activity was 7% higher. With respect to sensory traits, the traditional frozen products evaluated both before and after preparation for consumption slightly exceeded the quality of frozen asparagus obtained using the modified technology. Both types of frozen products prepared for consumption were evaluated at a level above 4 in a scale from 1 to 5. Compared with products stored at -20°C frozen products stored at -30°C usually showed a higher content of the analysed constituents, a higher level of the antioxidative activity and higher sensory quality.

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ZAWARTOŚĆ WYBRANYCH ZWIĄZKÓW PRZECIWUTLENIAJĄCYCH W SZPARAGU ZIELONYM W ZALEŻNOŚCI OD OBRÓBKI PRZED MROŻENIEM, CZASU I WARUNKÓW SKŁADOWANIA

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Oceniono zawartość związków przeciwutleniających w świeżym szparagu zielonym, szparagu przygotowanym do mrożenia oraz mrożonkach ze szparaga otrzymanych z wykorzystaniem technologii tradycyjnej, z materiału blanszowanego przed mrożeniem oraz mrożonek otrzymanych metodą zmodyfikowaną, ze szparaga ugotowanego. Gotowanie szparaga zielonego przed mrożeniem przyczyniło się do obniżenia zawartości witaminy C o 19%, polifenoli o 11%, karotenoidów o 9%, beta-karotenu o 6% i aktywności przeciwutleniającej o 18% (tab. 1). Szparag ugotowany, w porównaniu do szparaga blanszowanego, miał wyższą zawartość polifenoli, zbliżoną beta-karotenu, natomiast zawierał mniej karotenoidów, witaminy C i miał niższą aktywności przeciwutleniającą. Dwunastomiesięczny okres składowania mrożonki, w odniesieniu do oceny bezpośrednio po mrożeniu, spowodował średnio dla wszystkich prób obniżenie poziomu witaminy C o 22%, polifenoli o 10%, karotenoidów o 9%, beta-karotenu o 14% i aktywności przeciwutleniającej o 9% (rys. 1). Mrożonka ze szparaga zielonego otrzymana według zmodyfikowanej technologii i przygotowana do spożycia po 12 miesiącach składowania, przy podobnym poziomie witaminy C i beta-karotenu jak w mrożonce otrzymanej według technologii tradycyjnej, zawierała istotnie więcej polifenoli o 25% i karotenoidów o 10% oraz miała wyższą o 7% aktywność przeciwuteniającą. Pod względem cech sensorycznych mrożonki tradycyjne, oceniane zarówno przed jak i po przygotowaniu do spożycia, nieznacznie przewyższały jakością mrożonki przygotowane według zmodyfikowanej technologii (tab. 2, tab. 3). Obydwa rodzaje mrożonek po przygotowaniu do spożycia oceniono na poziomie powyżej 4 w skali pięciopunktowej. Mrożonki składowane w temperaturze -30°C miały z reguły wyższą zawartość analizowanych składników i wyższy poziom aktywności przeciwutleniającej, a także lepszą jakość sensoryczną niż mrożonki składowane w temperaturze -20°C.